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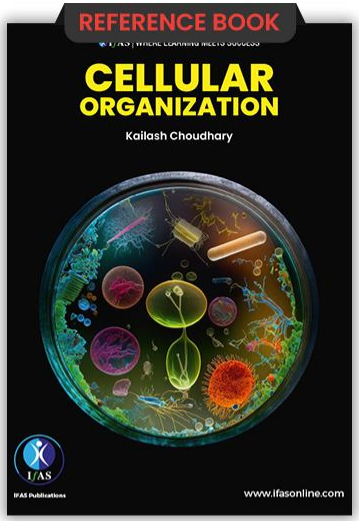
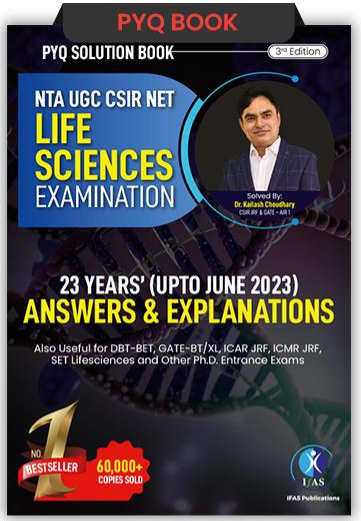
CSIR NET – Life Science

Unit 2: Cellular Organization

L14 Cell cycle

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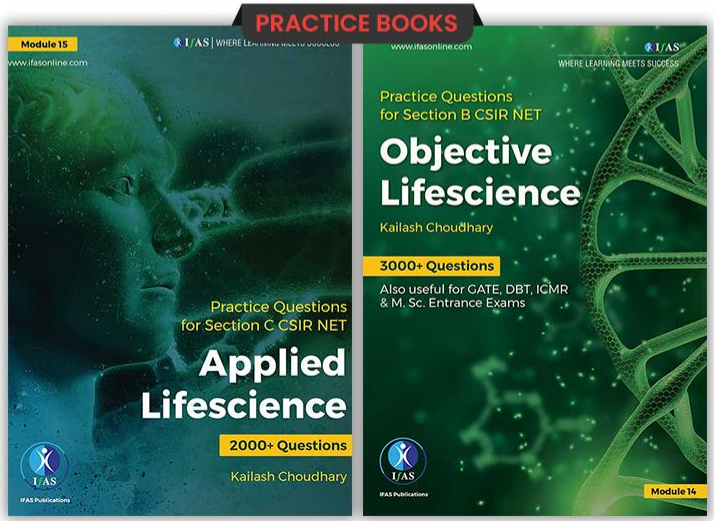

IfAS

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Points to be covered in this Lecture

- Bacterial cell cycle ✓
- Stages of eukaryotic cell cycle ✓
- Experimental approach to study cell cycle and function ✓
- Important regulatory proteins ✓

Cell Cycle

Cyclic event for DNA replication and cell division

Unicellular organism → division → Population growth

Multicellular organism → division → Growth of individual

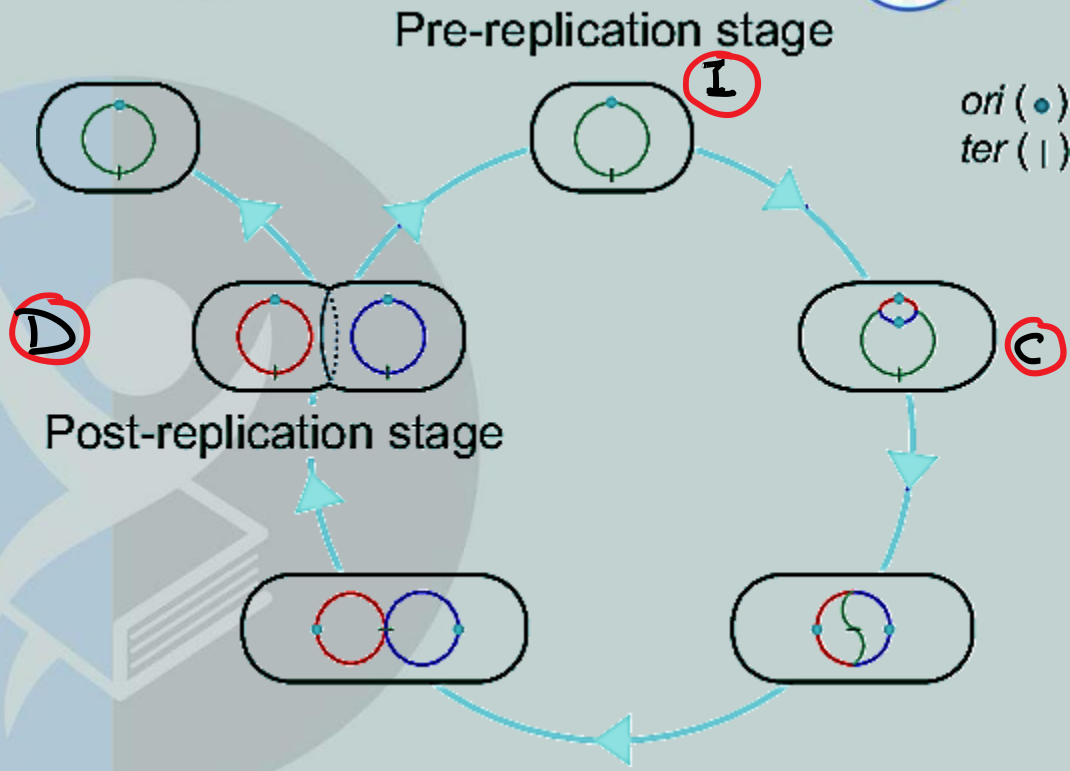
Bacterial cell cycle

C-phase, D-phase, and I-phase ✔ ✔ ✔

C= chromosomal duplication

D = division

I = interval (Negligible)



Overlapping between C and D-phase occurs

E.coli generation time 20 minutes ⇒ Division in every 20 min (during log phase)

Duration of C-phase = 33 minutes

Replication duration = $\frac{\text{genome size}}{\text{Replication speed} \times 2 \times 60}$

2 → Bidirectional Replication

E.coli genome size = 4×10^6 bp

replication speed = 1000 nt/sec

replication duration

Replication duration = $\frac{4 \times 10^6}{1000 \times 2 \times 60} = \frac{4 \times 10^2}{12}$

$= \frac{4 \times 100}{12} = \frac{400}{12} = 33.3$

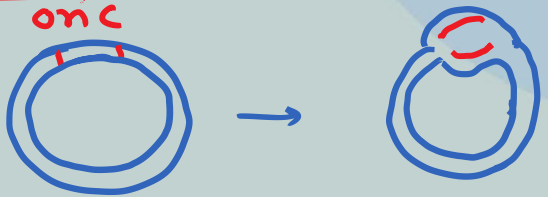
Replication duration = 33.3 min

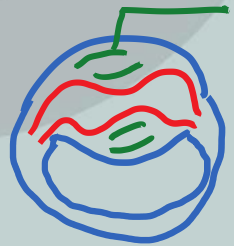
How generation time will achieve?

1. Overlapping of C and D-phase

- Chromosomal Duplication is overlapped with Division

2. Multiple round of replication



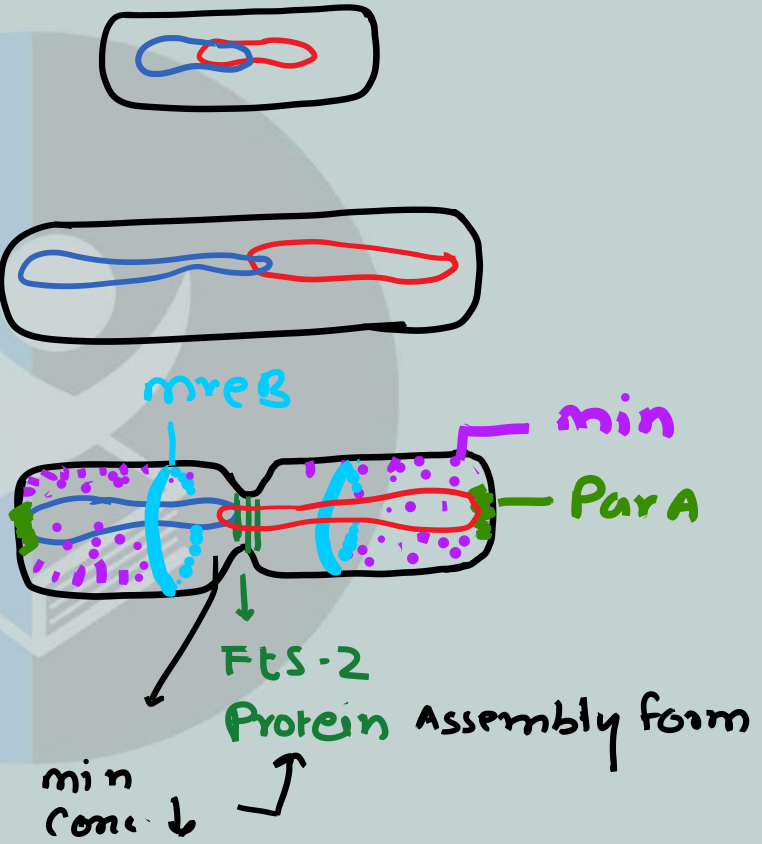


2nd Round Replication

- Start before completion of first Round.

- Ftsz protein = Filament temp Sensitive
- Min inhibits Ftsz assembly
- If min loss of function
- Minute sized cell formation
- Par A involved in chromosomal partitioning

- mre B = Control width of Bacterial cell

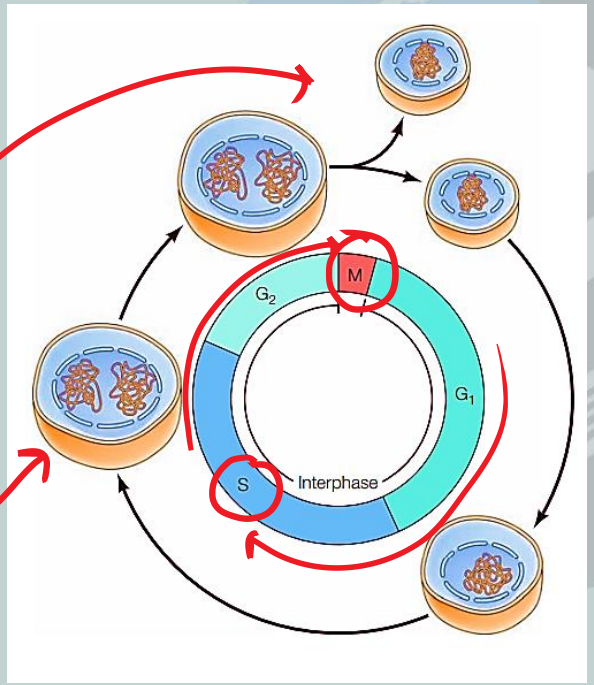


Eukaryotic Cell Cycle

Cyclic Event of Replication and Cell Division

Division

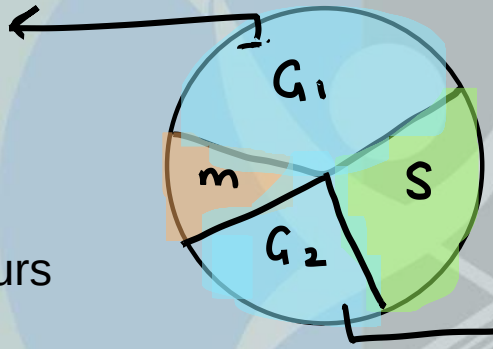
- Cytokinesis
- Karyokinesis



Replication S-Phase Synthesis Phase

- DNA Replication (Semiconservative)
- Early S-Phase = Euchromatin
- Late S-Phase = Heterochromatin
- Histone Synthesis
- Centriole Duplication

Cell Cycle Gap Phase (Preparatory Phase)



GAP-1 Phase / G1 Phase

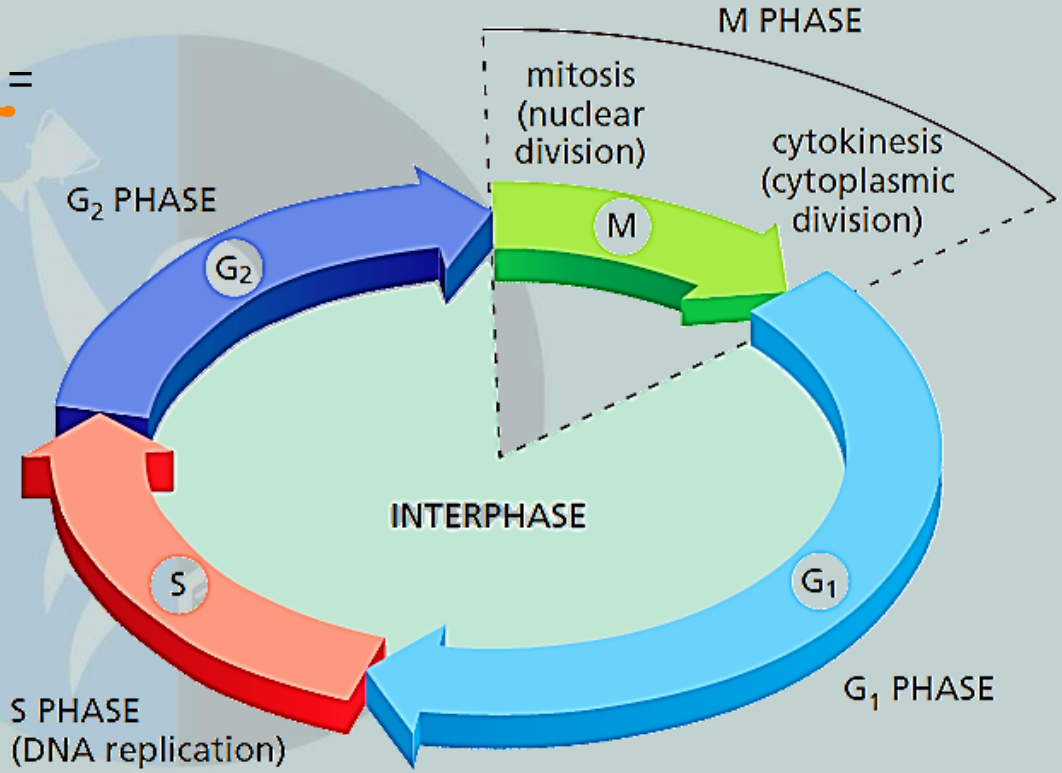
- Transcription Very High
- Translation Very High
- Organelle Duplication Occurs
- Nucleotide Biosynthesis

GAP-2 Phase / G2 Phase

- Tubulin Synthesis
- Centriole Division
- Transcription and Translation High

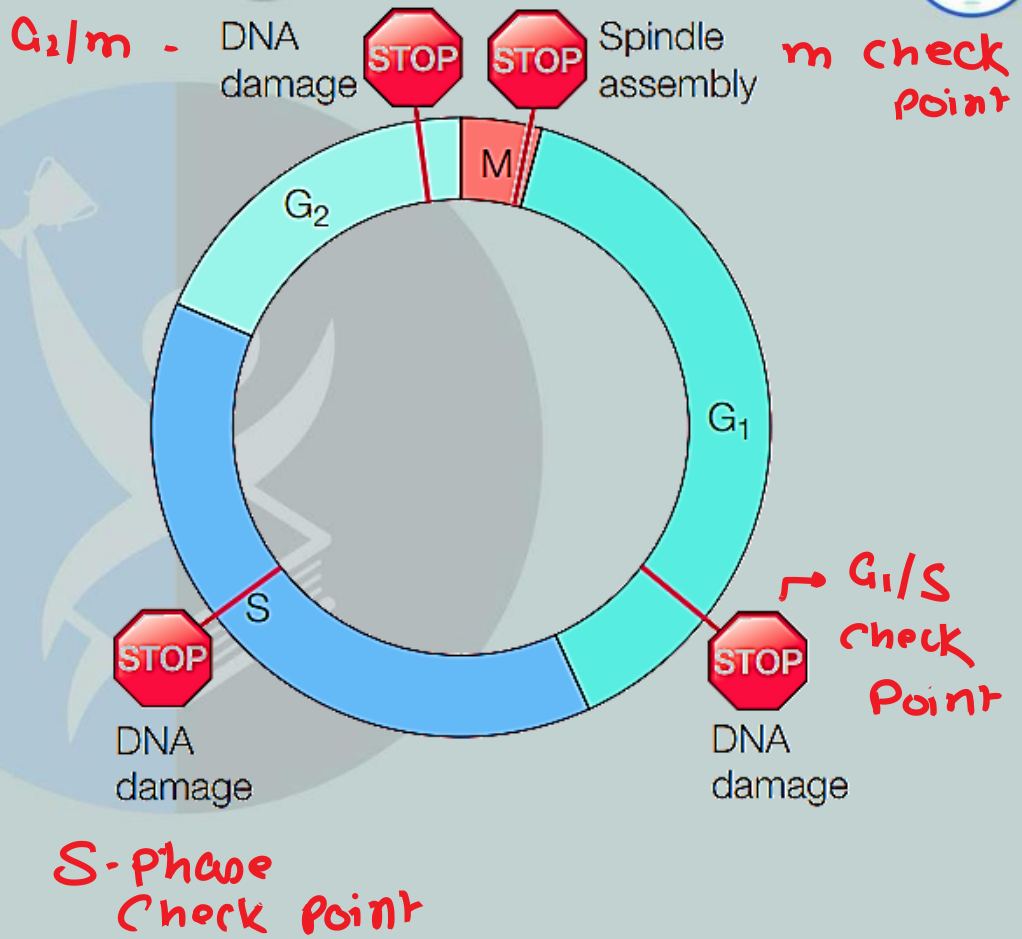
- G1, S, and G2 Phase - Interphase = Molecular Event Occurs

- M-Phase- Cytological Event Karyokinesis, Cytokinesis Occurs



Cell cycle checkpoints

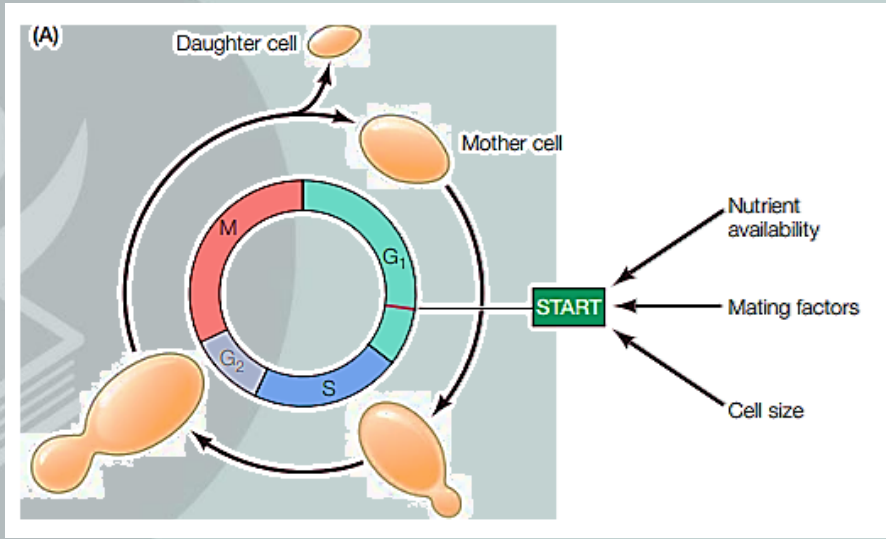
cell cycle checkpoints that prevent entry into the next phase of the cell cycle until the events of the preceding phase have been completed.



Check Point

G1/S Checkpoint, -

- major checkpoint in plants, animals, budding yeast
- known as restriction point or start point in *Saccharomyces cerevisiae* (budding yeast)
- G1S Checkpoint, check for –
- nutrient availability, space availability, DNA damage, growth factor.

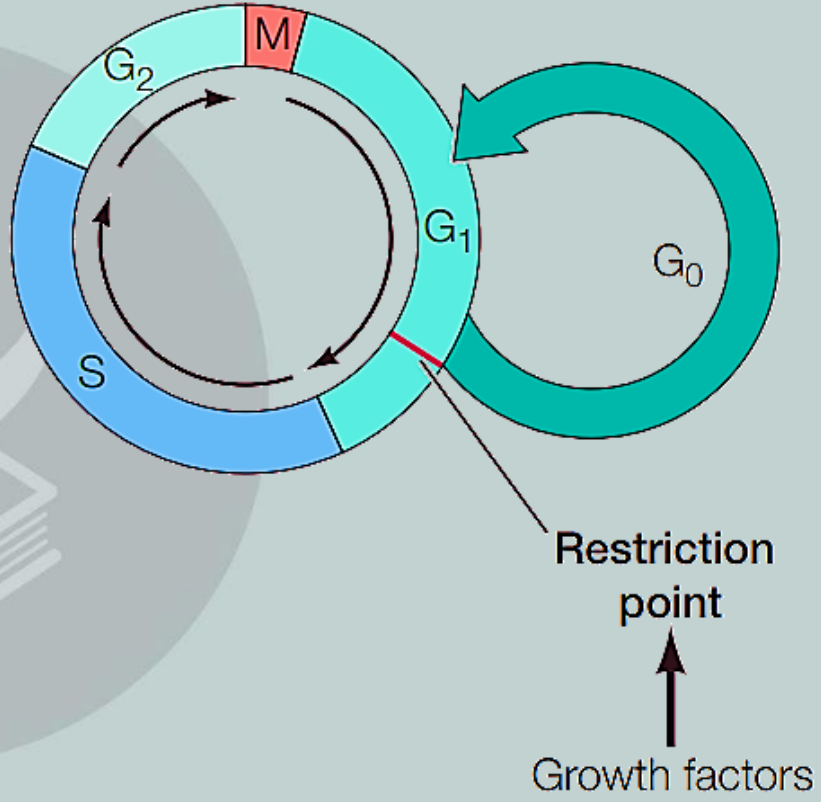


G0 phase/ quiescent phase-

- If all conditions are fulfilled, cell enters in S phase, otherwise cell arrested in G0 phase for indefinite time

✔in some cell, these conditions are never fulfilled, cell arrested in G0 phase for lifetime

i.e. terminal differentiated cells are arrested in G0 phase for lifetime i.e. skin, RBC, neuron



G2/M Checkpoint, start point – (major check point)

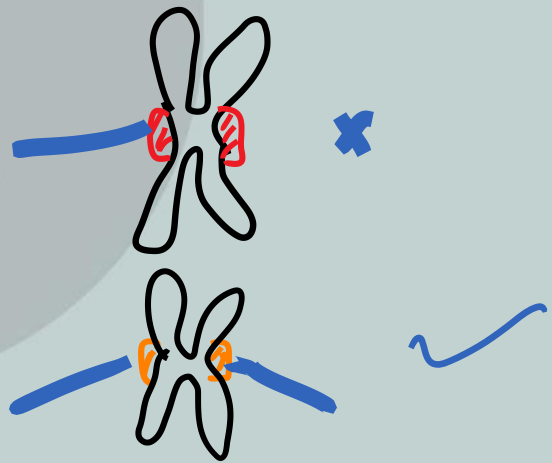
- *Schizosaccharomyces pombe* [fission yeast]
- in amphibians
- mammalian eggs(oocyte)

→ mammalian eggs(oocyte) arrested for long time in G2M Checkpoint,

S phase Checkpoint –

- repair of DNA damage,
- mismatched repair,
- temporary arrest,

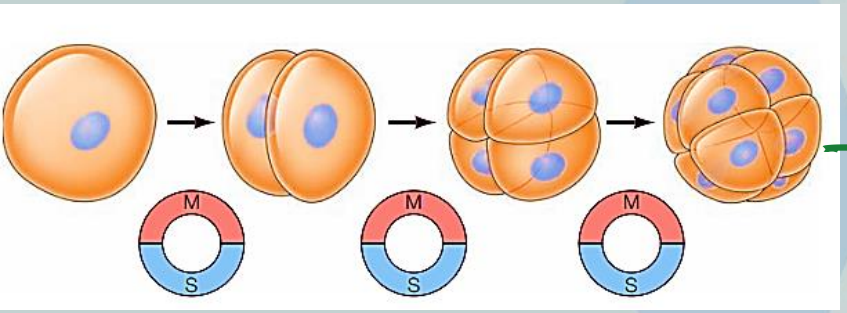
M phase Spindle Assembly Checkpoint, ensure correct association of spindle fibres with chromosome



. Unusual type cell cycle

Early embryonic stage -early cleavage division ⇒

Gap phase absent → S M



8-cell stage 16-cell stage

mid-blastula,

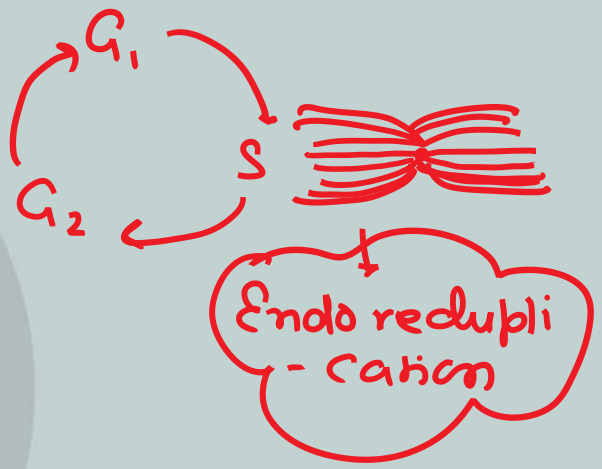
↓

Gap phase appear

- Transcription start.

Early cleavage division, fastest type of cell division

2. Chromosomal duplication without cell division



- Polytene chromosome formation

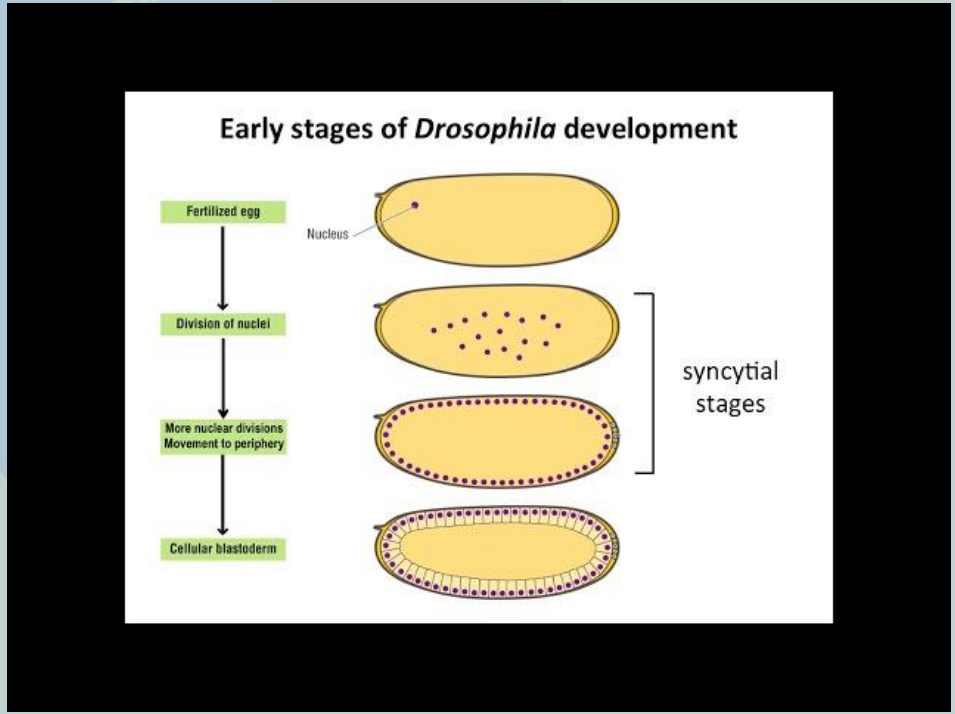
  Present in salivary gland of insect

- Larval stage of insect drosophila - 1049-chromated

- Chironemous larva - 4029-chromated.

✓ Cytokinesis absent

In case of Drosophila, early developmental stage - Syncytial blastoderm



Calculation of cell cycle duration

- Mitotic index

$$\text{Mitotic index} = \frac{\text{number of cells with visible chromosomes}}{\text{total number of cells}}$$

[m-phase]

Mitotic Definition

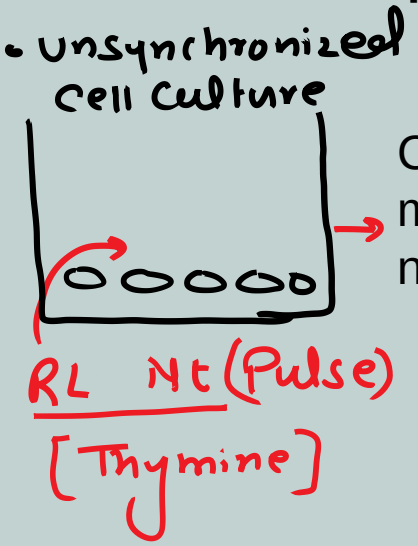
- The mitotic index is the ratio between the number of cells in mitosis in a tissue & the total number of observed cells.

- Formula:

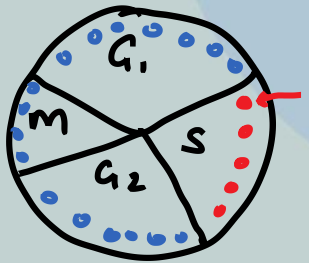
$$\text{Mitotic Index} = \frac{\text{Number of cells in Mitosis}}{\text{Total number of Cells}}$$

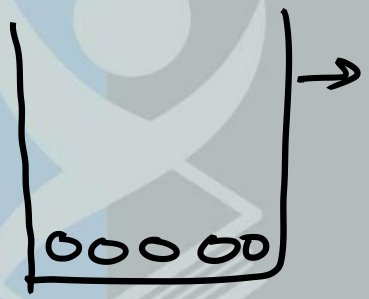
To find out duration of each phase of cell cycle, pulse chase experiment

Pulse chase experiment.



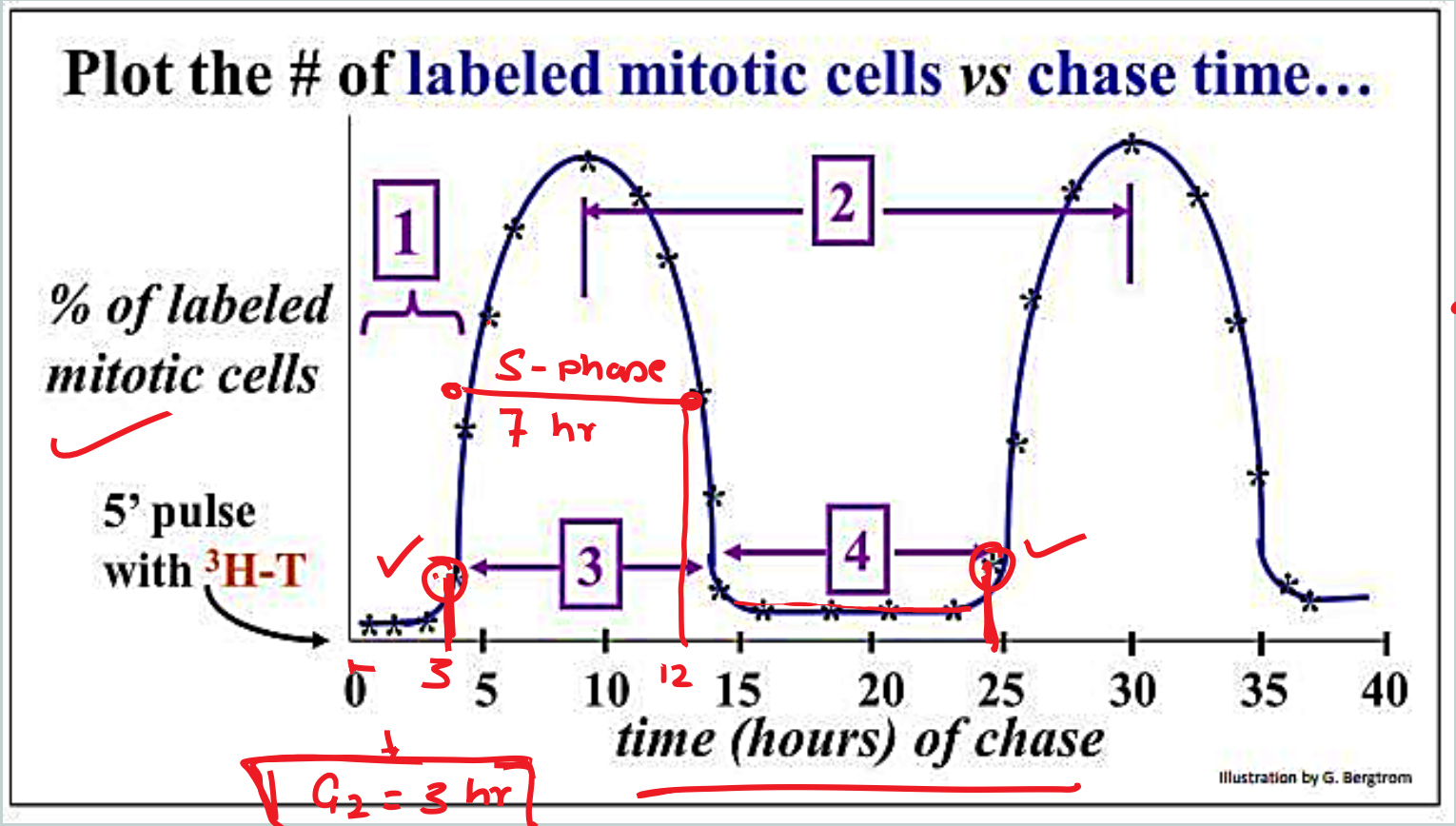
Cell replaced in fresh medium with normal nucleotide.





Chase radioactivity in M phase

- 0 minute no radioactivity
- 1 hour no radioactivity,
- 2 hour no radioactivity,
- 3 hour 25% radio labeled cell



3 hr = G_2

9 hr = S

25 - 3 = 22 hr

22 hr Total duration of cell cycle

Total duration 22 hour.

G2 phase = 3 hr

S phase = 7 hr

M phase = 1 hr

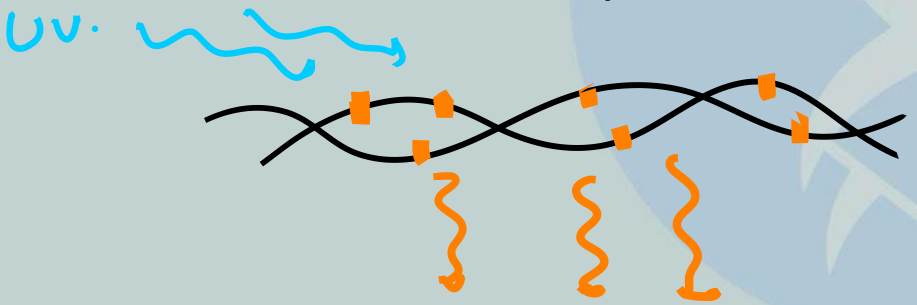
G1 phase = 11 hr

To find out which cell present in which stage of cell cycle - fluorescence activated cell sorting (FACS)

FACS is a type of flow cytometer

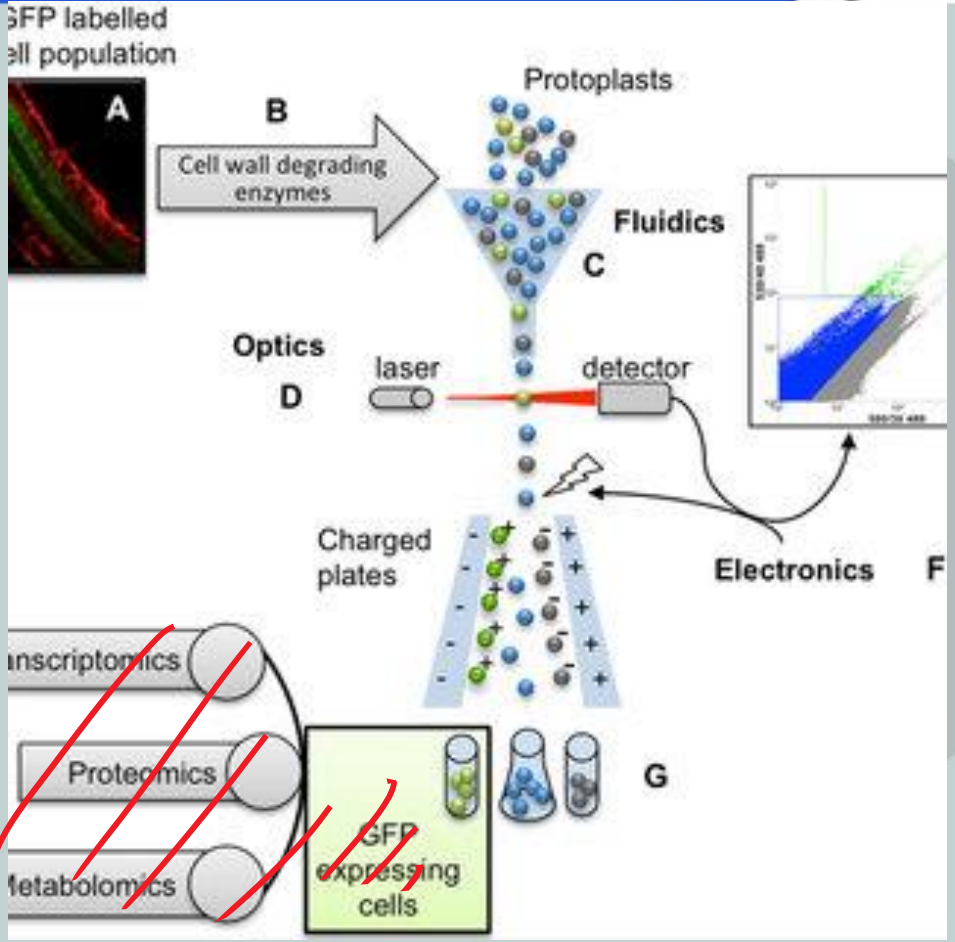
- on the basis of DNA content - DNA intercalating dye - intercalate in DNA → Fluorescence Emmition → Cell Sorting

Emission based experiment

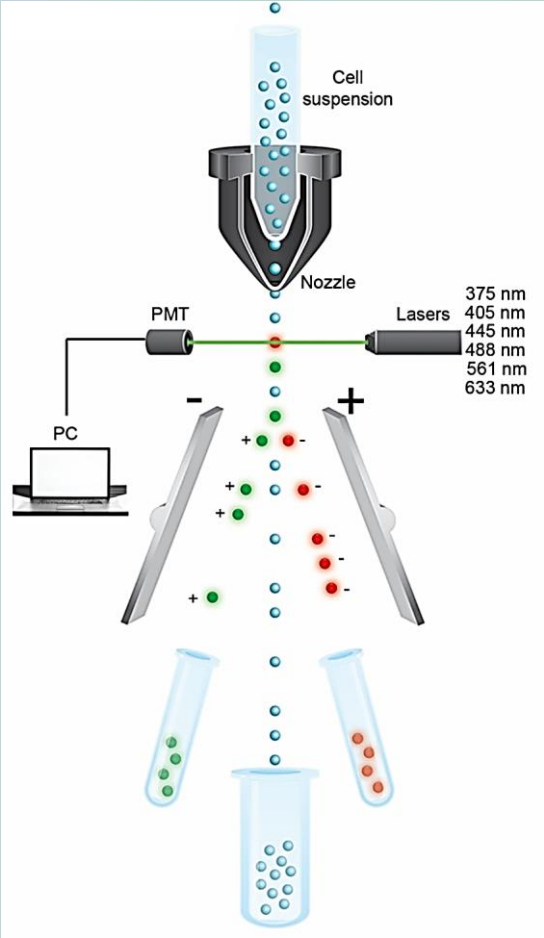


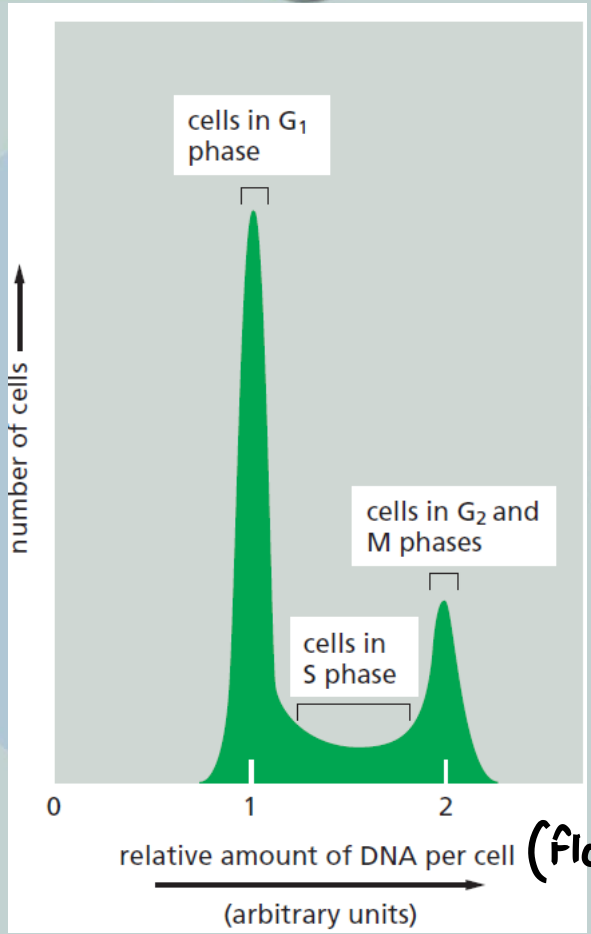
Fluorescence emission. → Based on DNA content

↑ DNA → ↑ Dye intercalation → ↑ Flourescence


GFP labelled
ell population
A
B
Cell wall degrading enzymes
Protoplasts
Fluidics
C
Optics
D
laser
detector
Charged plates
Electronics
F
nscriptomics
Proteomics
etabolomics
GFP expressing cells
G

	G1 phase,	S phase	G2 phase/M phase.
DNA content	2C	2C - 4C	4C
fluorescence.	1	1 - 2	2



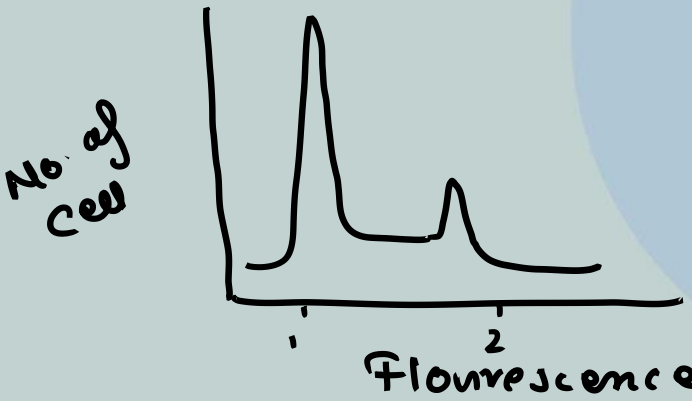


(fluorescence)

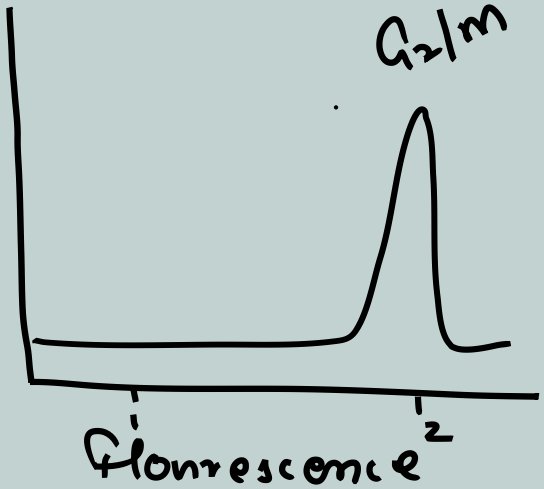
Que

Cell culture unsynchronized

Control
↓
FACS



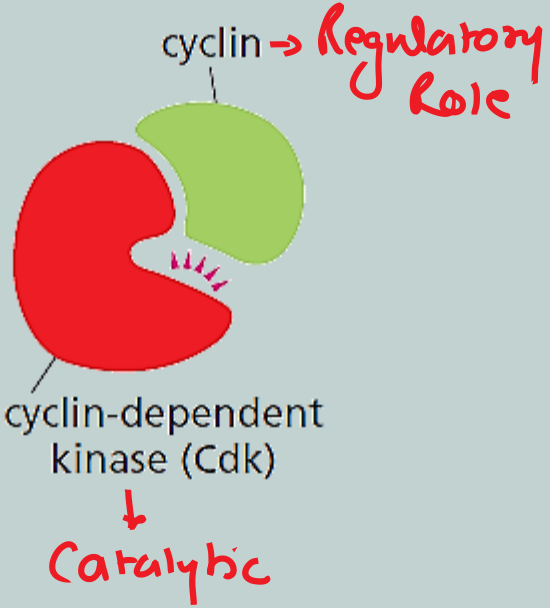
Drug treatment



Cell Cycle Regulatory Protein

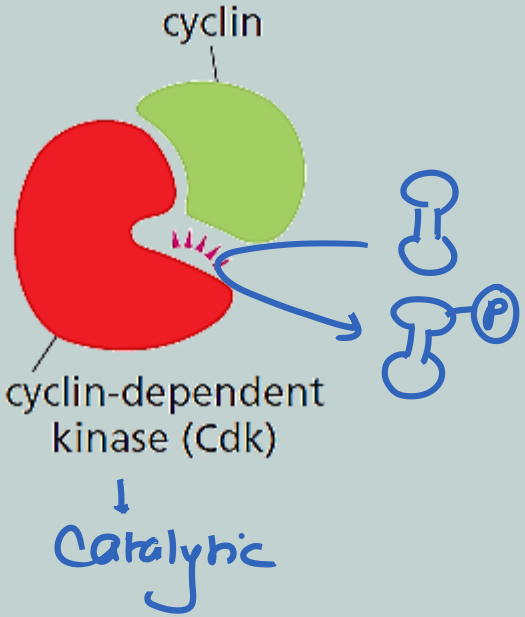
Cycline - works in association with CDK

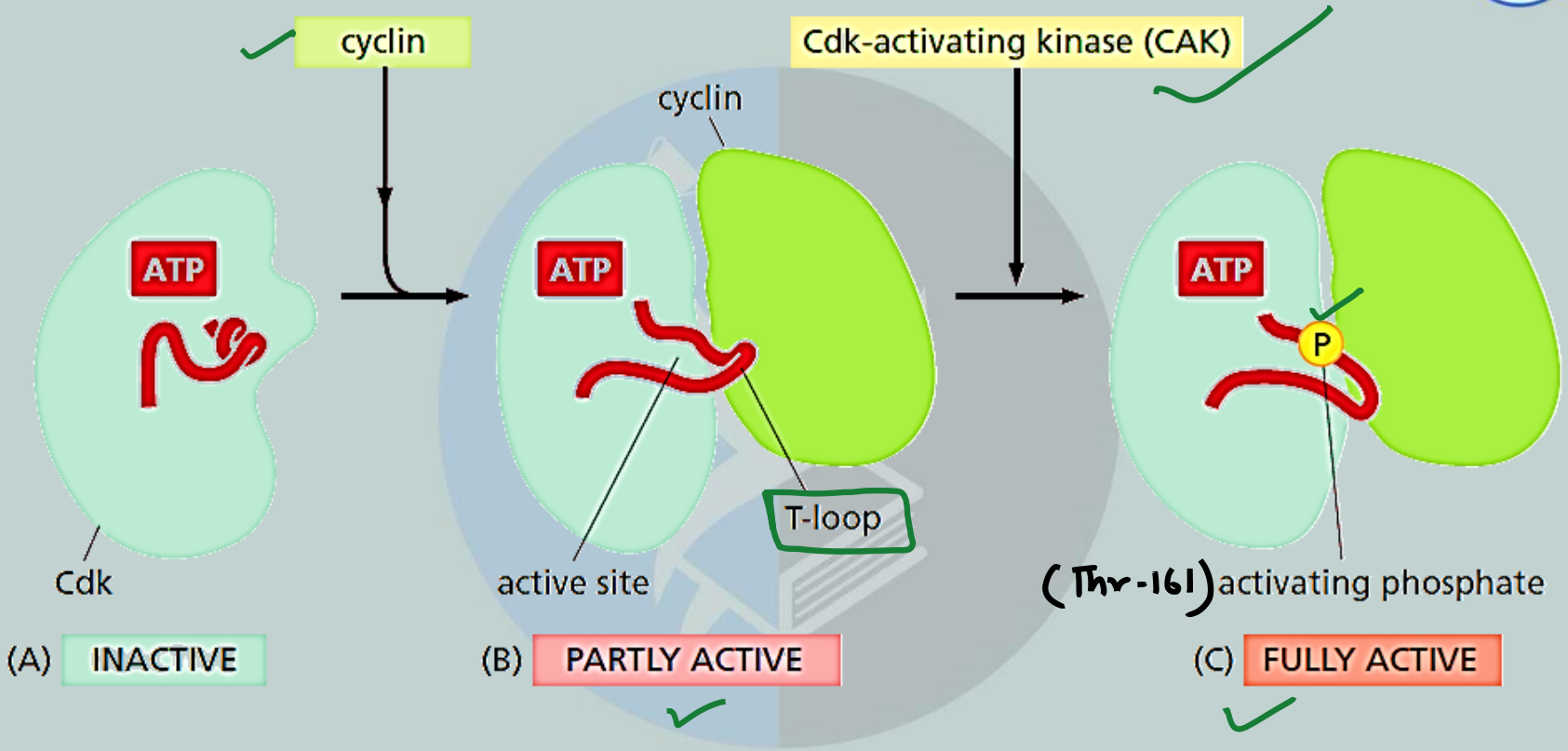
- Cyclic Fluctuation, can be seen during different phase of cell cycle.
- Different type of Cycline works in different phase of cell cycle.
- Cyclic Fluctuation means synthesis and degradation by polyubiquitinization



2 CDK - Cycline Dependent Kinase

- CDK, Catalytic Subunit,
- add phosphate on its target,
- binds with cycline




cyclin
Cdk-activating kinase (CAK)
cyclin
ATP
ATP
ATP
P
T-loop
Cdk
active site
(Thr-161) activating phosphate
(A) INACTIVE
(B) PARTLY ACTIVE
(C) FULLY ACTIVE

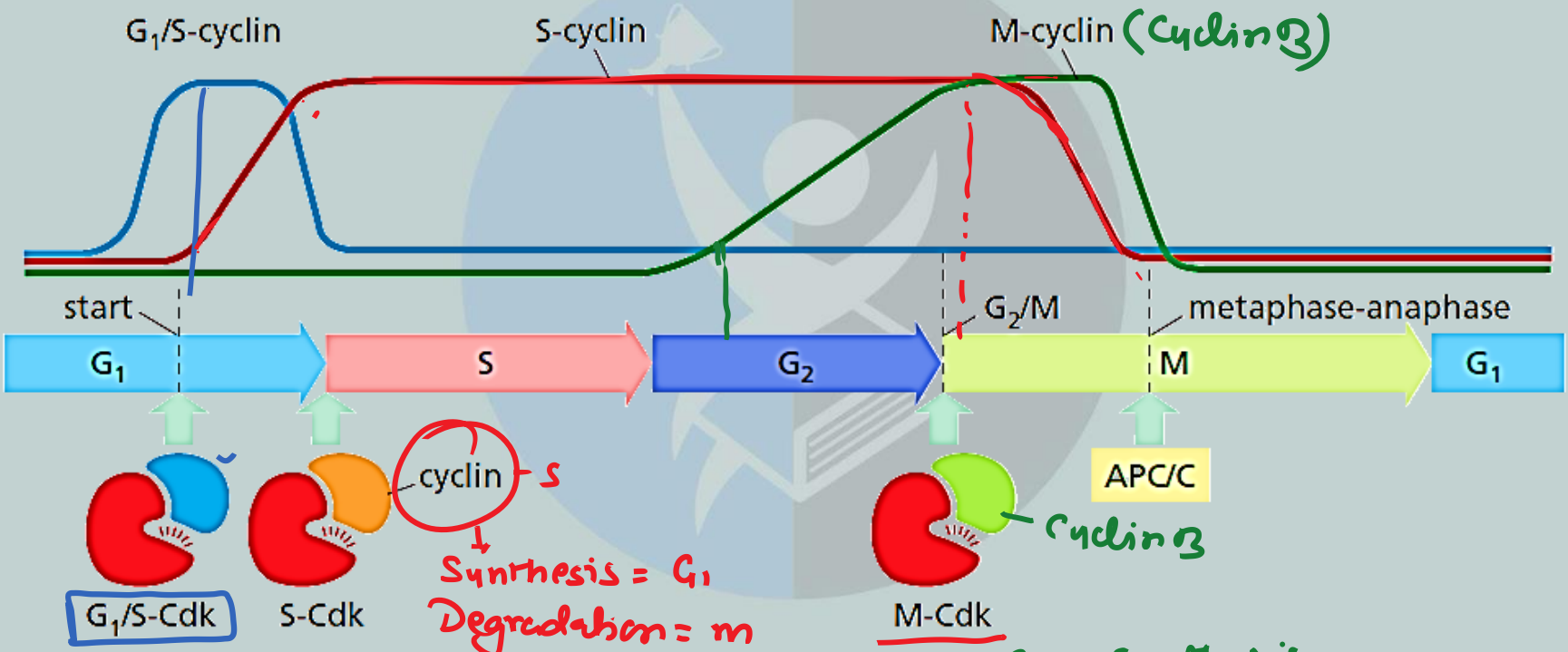
gmp

The Major Cyclins and Cdks of Vertebrates and Budding Yeast

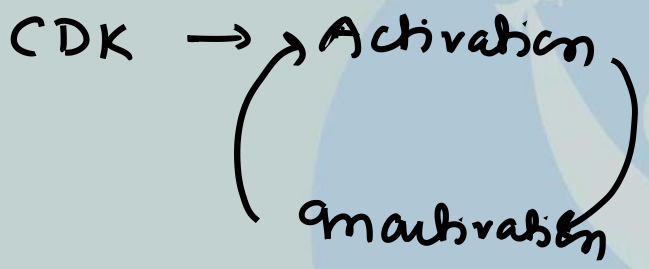
	Vertebrates		Budding yeast	
Cyclin–Cdk complex	Cyclin	Cdk partner	Cyclin	Cdk partner
G_1-Cdk	Cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	Cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	Cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	Cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

#Cyclic Fluctuation in Cyclin during different phase of Cell Cycle

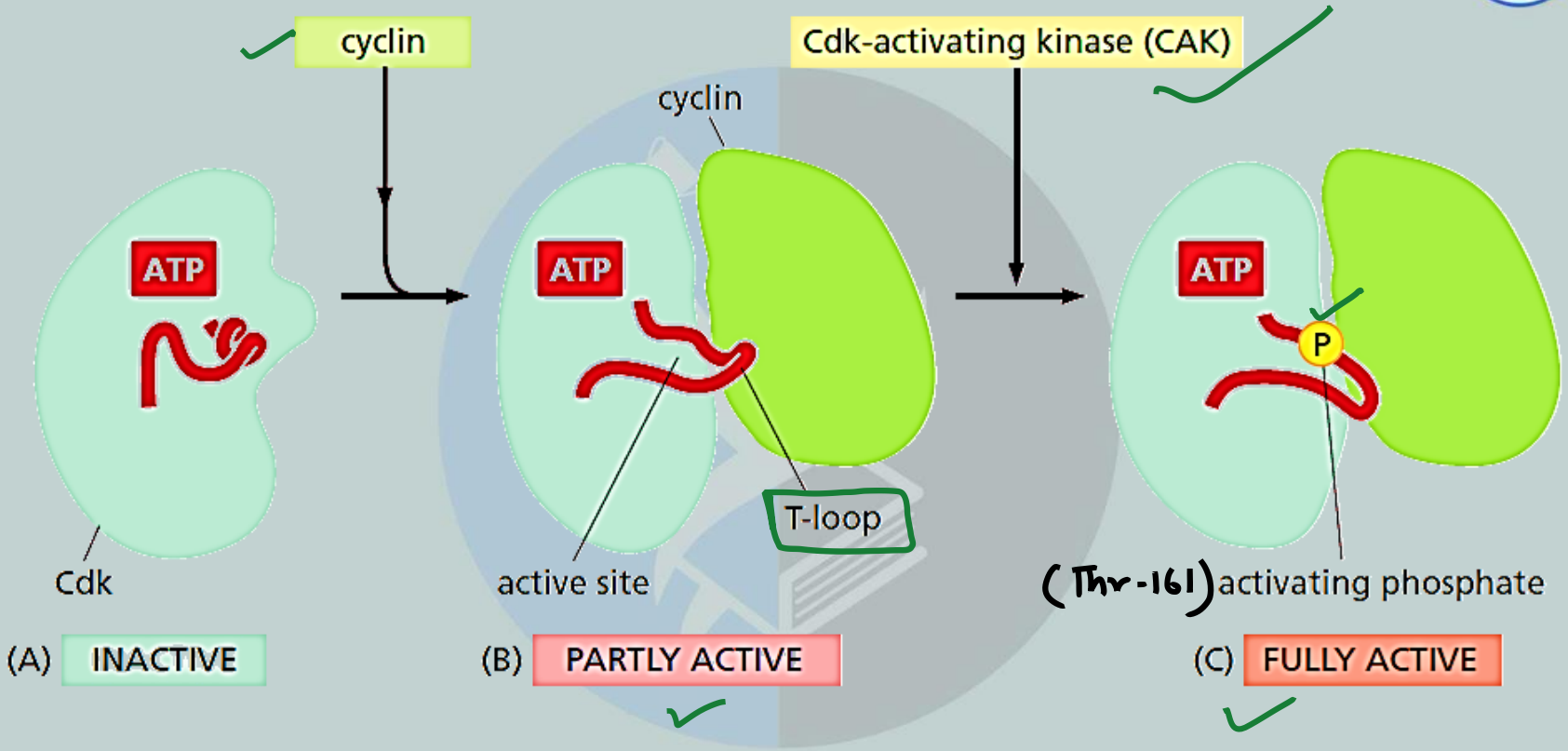


→ CDK level remain same during different phase of cell cycle



3 CDK, Activatory Kinase (CAK)

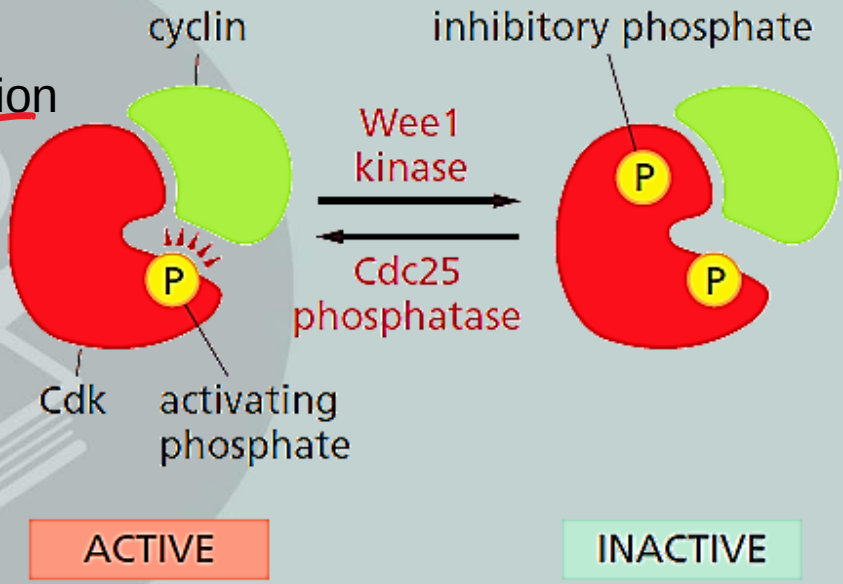
- CAK, add phosphate on CDK, (Thr 161) → Activating position
- activate CDK by phosphorylation on CDK.
- CAK - (CDK7 + Cycline H)
- work in transcription (Helicase activity & kinase activity)
- Multiple subunit kinase activity helps in replication and repair


cyclin
Cdk-activating kinase (CAK)
cyclin
ATP
ATP
ATP
P
T-loop
Cdk
active site
(Thr-161) activating phosphate
(A) INACTIVE
(B) PARTLY ACTIVE
(C) FULLY ACTIVE

4. CDK inhibitory kinase – (CIK)-

add phosphate on inhibitory position of CDK,

threonine-14 and tyrosine-15. Inhibitory position (dual specificity kinase to target threonine-tyrosine).



Wee 1 kinase inhibits Cdc2 fission yeast

Dual specificity phosphatase.

E.g. Cdc25 phosphatase activates Cdc2 by removal of inhibitory phosphate.

6. Kinase Inhibitory Protein (KIP) And Cyclin Inhibitory Protein (CIP).

KIP binds with CDK and prevents its association with cyclin. P15, P16, P18, P19,

INK4 - inhibitor of kinase 4, CDK4,

P21 inhibits CDK2,

INK2 inhibitor of CDK2.

CIP E.g. P27 inhibits cyclin E

In yeast Far 1 inhibits Cdc2,

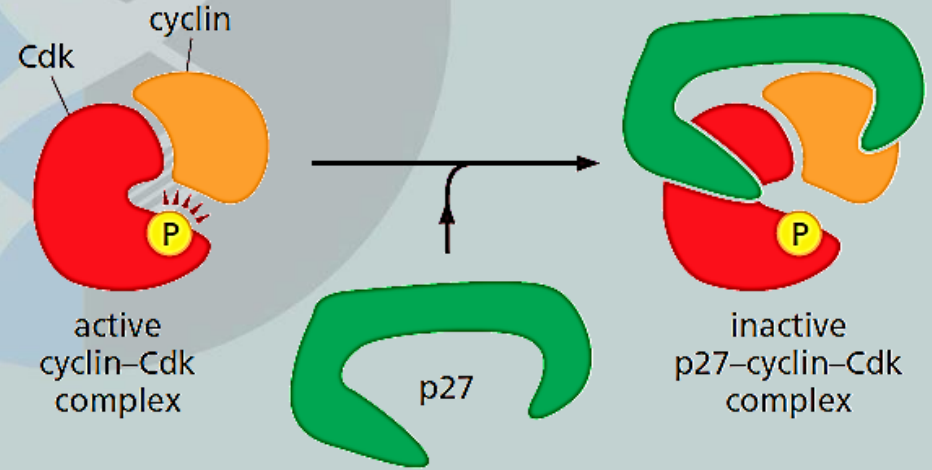
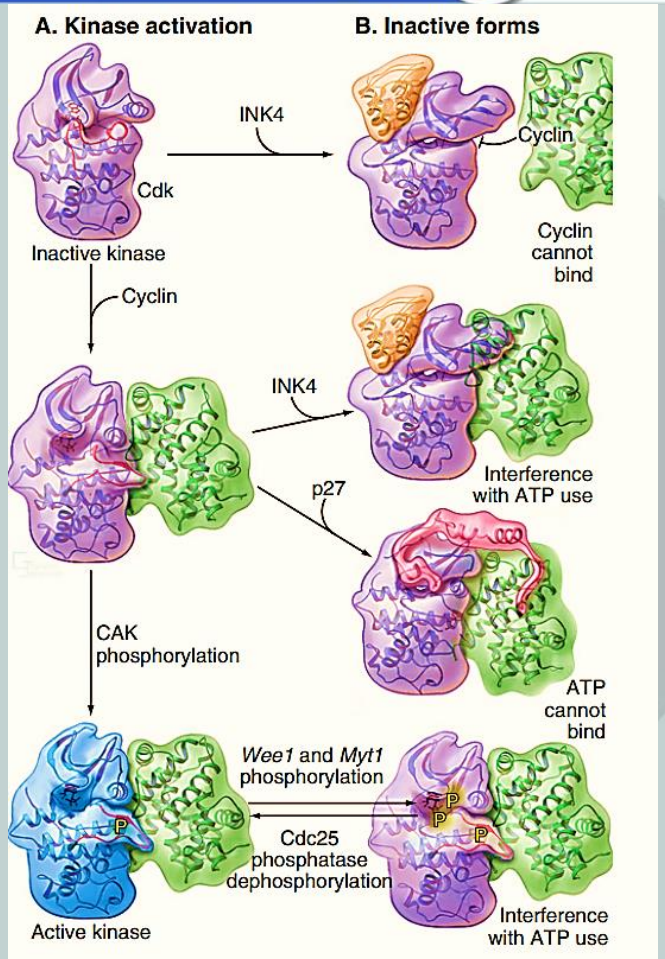


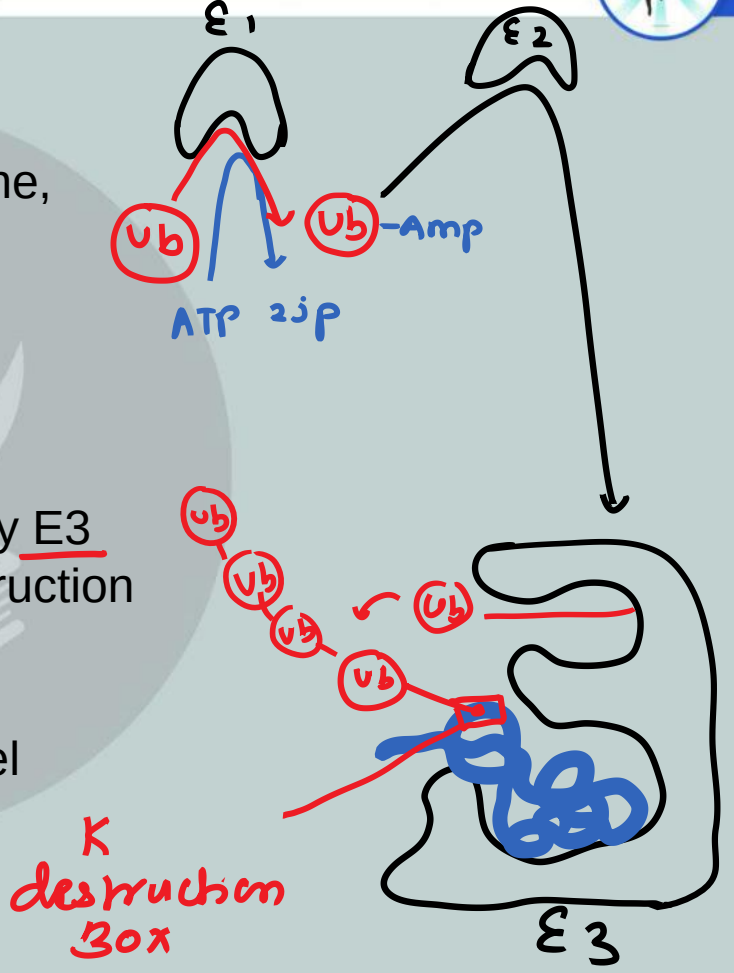
Table 18.1 Cdk Inhibitors

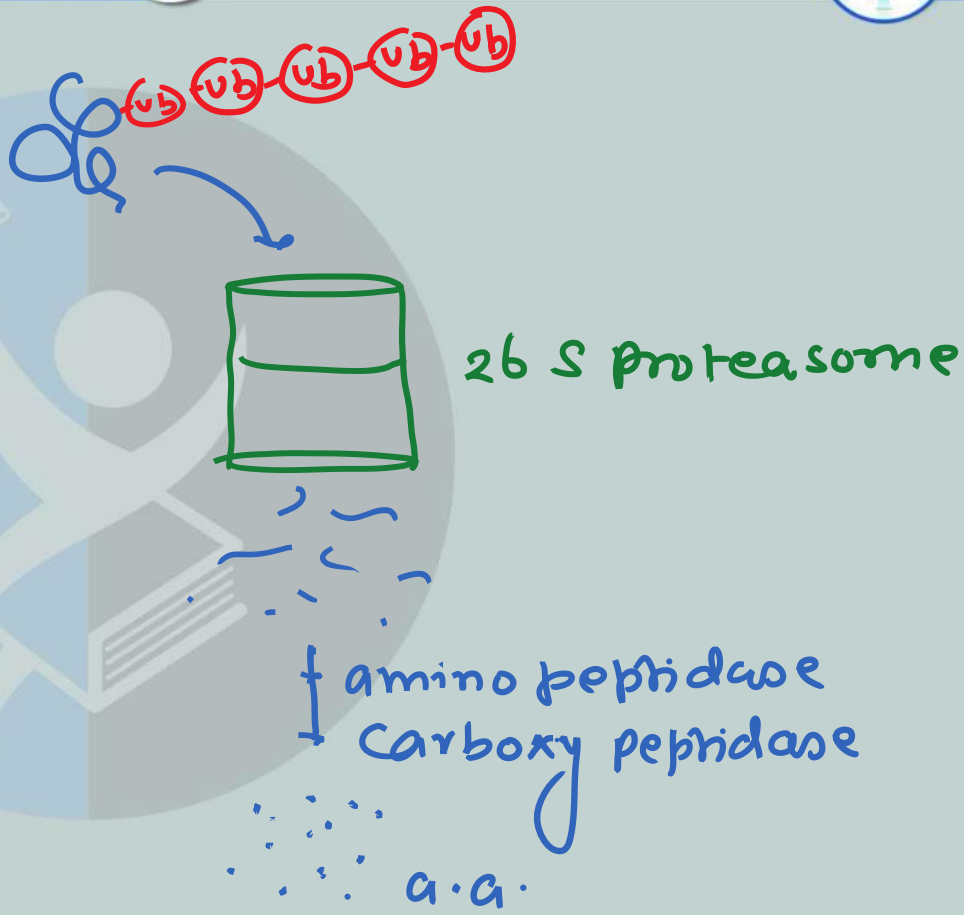
Inhibitor	Cdk or Cdk/cyclin complex	Cell cycle phase inhibited
Ink4 family (p15, p16, p18, p19)	Cdk4 and Cdk6	G_1
Cip/Kip family (p21, p27, p57)	Cdk2/cyclin E	G_1
	Cdk2/cyclin A	S, G_2


A. Kinase activation
B. Inactive forms
INK4
Cdk
Cyclin
Inactive kinase
Cyclin
Cyclin cannot bind
INK4
Interference with ATP use
p27
CAK phosphorylation
ATP cannot bind
Wee1 and Myt1 phosphorylation
Cdc25 phosphatase dephosphorylation
Active kinase
Interference with ATP use

7. Ubiquitin ligase –

- F-box protein, conserve F-box sequence in gene,
- enzyme complex of E1, E2, and E3,
- E3 is actual ubiquitin ligase.
- Ubiquitin is a protein of 76 amino acid added by E3 ubiquitin ligase on lysine residue of lysine destruction box on target protein.
- Lysine destruction box, 26S proteasome, model organism for cell cycle study




ub
ub
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26 S proteasome
amino peptidase
Carboxy peptidase
a.a.

Model Organism for Cell Cycle Study Eukaryote

Yeast *Saccharomyces cerevisiae* -Budding yeast

schizosaccharomyces pombi - Fission yeast

Advantages - Simple Eukaryote, Easy cultured in lab

Less number of regulatory genes are present

Short life span = 90 min

Natural conditional mutant are available

Conditional mutant

Act as wild type in permissive condition

Act as mutant in non-permissiv

act as mutant in specific condition (Non-permissive)

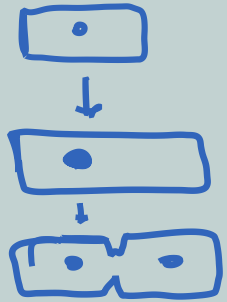
eg -

- Temperature sensitive ✓
- Light sensitive ✓
- pH sensitive ✓
- Heavy metal sensitive Yeast ✓

Wee 1 kinase ts

Permissive condition

25 degree centigrade

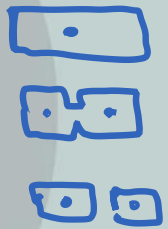


cell Normal-sized cell

(wild type)

Non-permissive condition

35 degree centigrade



Wee-sized cell / Small-sized

CDC-25 phosphatase (Temperature sensitive)

Permissive condition

Normal-sized

Non-permissive condition

Cdc 25 Phosphatase Non functional

Elongated cells Large-sized

Inhibitory (P) can not removed

↓

Cell keep on Elongated

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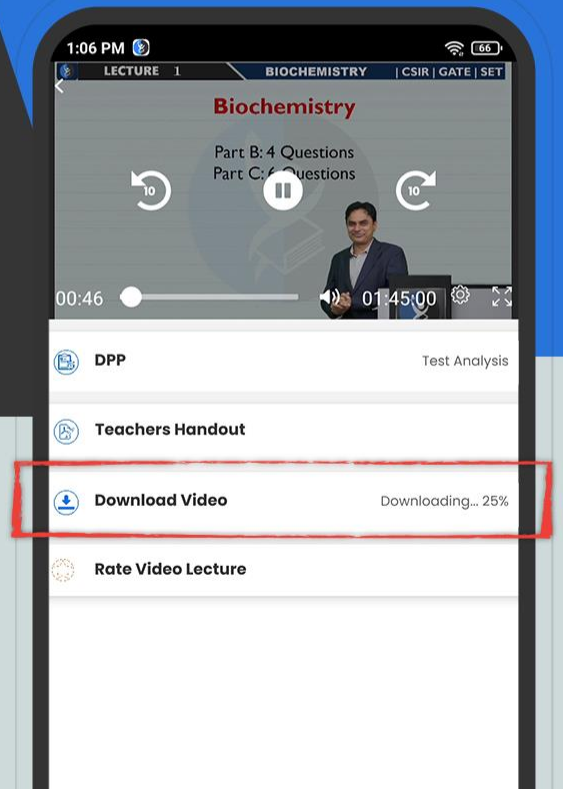

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